Quick Reference Guide on USDA Swine Influenza Virus (SIV) Surveillance

SIV Surveillance Detailed Sample Collection Procedures

This document (an excerpt from the SIV surveillance manual) explains which samples are to be collected and the procedures to follow for surveillance sample collection. All collection procedures in this section are validated for SIV diagnostic work and validated for SIV surveillance activities.

A. Tools Needed

- For removing lung tissues from euthanized or expired animals
  - Knife and scissors
  - Forceps
  - Plastic Whirl-Pak® bags or screw top-plastic tubes

- For collection of nasal swabs
  - Dacron/polyester or synthetic nasal swabs (no wooden handle swabs; no cotton swabs)
  - Collection tube with viral transport media [such as Tris Buffered Tryptose Broth (TBTB) or Brain Heart Infusion (BHI)]
  - Hog snare or other swine restraining device
  - Ear plugs

- Tools and items necessary for both lung tissues and nasal swab collection
  - Cooler
  - Ice packs
  - Fine-point permanent marker or other waterproof marker
  - Ball-point pen
  - Pan or bucket for disinfecting instruments after appropriate contact time to ensure disinfection
  - Bleach (disinfectant)
  - Personal protective equipment (PPE) to protect yourself from potential exposures
  - Trash Bags for soiled PPE
  - Submission form(s) - VS Form 10-4 for regulatory officials (http://www.aphis.usda.gov/library/forms/pdf/VS_Form10_4.pdf) or Appendix F from SIV Surveillance Plan which can be found here: http://animalhealth/surveillance/siv/SIV%20Procedures/2010%20July%20Influenza%20Surveillance%20in%20Swine%20Procedure%20Manual%20Appendix%20F%20Submission%20Form%20for%20Regulatory%20Veterinarians.pdf

- If bar code labels are needed, contact NVSL Shipping. Contact information for NVSL appears on page 2 of the SIV Surveillance Manual: http://animalhealth/surveillance/siv/SIV%20Procedures/2010%20July%20Influenza%20Surveillance%20in%20Swine%20PROCEDURE%20MANUAL.pdf.

B. Steps in Collecting Specimens for SIV

Nasal swab or lung tissue should be taken for SIV surveillance testing.

Nasal swab: DO NOT pool swabs from individual pigs.

- The pig should be properly restrained with the head positioned upward to allow easy access to the nasal cavity. Anesthesia is not needed.
- Insert a sterile Dacron/polyester swab into the nasal cavity and gently swab the surface of the nasal mucosa, using a circular motion to cover as much of the nasal mucosal surface as possible. Avoid touching the skin with the swab as you enter the nasal cavity.
- The swab will collect nasal mucosal secretions and surface epithelium. It is important not to scrape too hard, as drawing blood is undesirable.
- Remove the Dacron swab from one nostril and repeat the same procedure in the other nostril, using the same swab.
- Once the nasal swab (both nostrils) has been collected, mix the swab in a transport media designed for maintaining viruses (TBTB or BHI viral transport media).
- The volume of viral transport media should be sufficient to cover the head of the swab.
- To remove the swab handle, back the swab out of the tube slightly and bend the portion of the handle that the collector touched back and forth over the edge of the tube until it breaks. This portion of the handle is considered contaminated and should be discarded. Alternatively, scissors or wire cutters may be used to cut the swab handle.
- Clearly label with appropriate ID (using a waterproof marker), place the sealed tubes in a Whirl- Pak® bag, and immediately refrigerate or chill.
- Store and ship in an upright position to reduce chances of leakage.

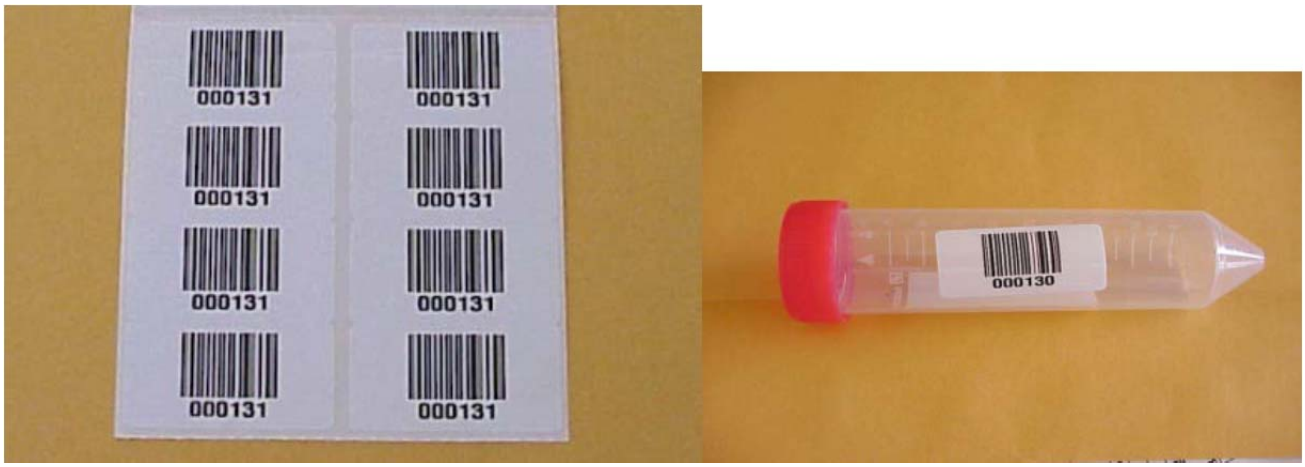
Lung Tissue

- When collecting lung tissue, the tissue should be fresh, or as fresh as possible. Collect multiple sections of lung tissue from affected areas.
- Samples should be **at least** half-dollar size.
- Be sure to include the junction of normal and abnormal lung tissue.
- Double bag, using Whirl-Pak® bag for at least the inner bag, clearly label with appropriate ID (using waterproof marker), and immediately refrigerate or chill.
- Lung tissue from each individual animal should be kept in a separate bag.

C. Proper Labeling of Samples

Samples should be labeled with bar code labels, if available.

- Label each tube or sample collection container with a waterproof pen. Include on each label:
    - Sample number
    - Type of specimen in tube or container (i.e., lung tissue, nasal swab)
    - Bar code identification label should be used if available
        - Bar codes are printed in sets of 4 individual labels. Each sample should receive a **different** bar code, even if several samples are collected from the same animal.
        - Bar codes should be used as follows:
            - One label on each sample tube – be sure to place bar code **lengthwise** along the tube.
            - One label on the submission form
            - Any labels that are not used should be destroyed



- Place the sealed samples in a Whirl-Pak® bag, and then place in a cooler and/or on cold packs. **Do not freeze specimens**.
- Properly dispose of non-submitted tissues and/or carcass.
- Send the samples overnight to a participating NAHLN SIV testing lab or NVSL.
- Questions regarding sampling techniques can be directed to the program managers at APHIS-VS-National Center for Animal Health Programs (NCAHP). Contact information for NCAHP appears on page 2 of the SIV Manual: http://animalhealth/surveillance/siv/SIV%20Procedures/2010%20July%20Influenza%20Surveillance%20in%20Swine%20PROCEDURE%20MANUAL.pdf..

SIV Diagnostics Summary Information

- NAHLN labs must use standardized assays for USDA SIV surveillance. NVSL uses the same standardized assays. This means we are comparing apples to apples when you look at the NAHLN lab results and NVSL results.
- The NAHLN labs differ in the type of tests they have chosen to and approved to run for SIV surveillance
    - Go to this website to know what your lab is approved to do. Recognize that some labs are still working to implement the subtyping PCRs (see below). http://www.aphis.usda.gov/animal_health/nahln/downloads/siv_lab_list.pdf
- NAHLN labs do not have to use the NAHLN assays if they are testing samples that are not going into the SIV surveillance stream. If they are testing only for the practitioner's needs, they may use the NAHLN assays or they may use their own assays.
- The matrix PCR is the screening PCR (first test run on a suspect SIV case). If it is positive, it means influenza nucleic acid is present. It doesn't tell us what influenza is present.
- NAHLN labs have recently (within the last 2 months) been offered the option to run the subtyping PCR's (2). One tells the H type (H1 or H3) and the other tells the N type (N1 or N2).
- In the current algorithm, labs do H, N, and M gene sequencing either from the original sample material or when they have grown a virus in cell culture. Prior to having the subtyping assays, the only way we could tell the H and N types was to look at the sequence. The subtyping PCR's can speed up obtaining the H and N types as we don't have to wait several days for virus to grow and sequencing to occur.

- NVSL has access to what we call the differential or pandemic matrix PCR. This PCR specifically detects the matrix gene that was found in the pandemic H1N1 as this gene was unique to what we had circulating in pigs at the time it was introduced to U.S. swine. Like pandemic N1 it also has moved into our endemic viruses, resulting in our current H3N2 issue.
- Samples to be referred to NVSL include:
    - samples associated with human cases;
    - samples that require sequencing (if not to be done in the NAHLN laboratory);
    - and samples for which the results are inconclusive or unusual.

Key points – pertains ONLY to USDA SIV surveillance testing in NAHLN laboratories

- Matrix positive only means flu is present
- H3N2 based on subtyping means only that we have H3N2 virus. It doesn't tell us if the pandemic matrix gene is present.
- The only way to tell if H3N2 has the pandemic matrix is to sequence the M gene or use the differential/pandemic matrix PCR (in use at NVSL)
- If provided only a N1 result, ask which test was used. Don't assume it was the subtyping PCR; previously NAHLN laboratories were using the N1 PCR, which was a different assay
- PCR results are reported as Ct's. The lower the number (closer to 1) the more nucleic acid is present.
- Larger numbers (higher Ct's) mean less nucleic acid. With high Ct's we may not be able to grow virus and/or we may not be able to get a result (or have incomplete results) for the subtyping PCRs and/or may not be able to get a result for the differential matrix PCR.
- Higher Ct's also mean we may not be able to directly sequence from the nasal swab or lung material. We are still trying to determine where that matrix threshold is for direct sequencing, but in general if the CT is in the 20's we feel we have a good chance of getting at least some sequence. If the CT value is in the 30's, it might not be worth pursuing and instead try to grow virus and then sequence the virus.
- NVSL's Diagnostic Virology Laboratory should be contacted about and inconclusive results and prior to sample.

Timeline

- Matrix and subtyping PCR's can be accomplished in a single day (see algorithm for defined times) http://www.aphis.usda.gov/animal_health/animal_dis_spec/swine/downloads/revised_appendix_c_testing_guidelines.pdf
- Virus isolation can occur within a couple of days of starting the assay, but can take up to 2 weeks if a slow growing virus. Generally picked up in under a week for most infections.
- Sequencing results can be highly variable in availability-depends on amount of nucleic acid, quality of nucleic acid, and if full gene sequence is obtained or if gaps have to be filled in with additional testing.

Payment

- NAHLN laboratories participating in USDA SIV surveillance testing have agreements in place to be reimbursed for all testing associated with the SIV algorithm.